EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 12
Issue: 2
Year: 2025

EFFECTS OF ETHANOLIC EXTRACTS OF AZADIRACHTA INDICA, CYMBOPOGON CITRATUS AND OCIMUM GRATISSIMUM ON THE HAEMATOLOGICAL INDICES, FOLLOWING PLASMODIUM BERGHEI-INDUCED MALARIA IN MICE

Ezeokafor Emmanuel Nonso[1], Nwankwo Azubike A.[2], David Chibuike Ikwuka[3], Ifemenam Kosisochukwu Emmanuel*[4], Ifebi Kenechukwu Onyeka[5], Dike Charles Chijioke[6], Onoriode Akpghene Eyeghre[7], Uzoh Casmir Ifunanya[8] and Ottih Chidalu Jennifer[9]

[1,7]Department of Human Physiology, Faculty of Basic Medical Science, Nnamdi Azikwe Univerisity, Nnewi.
[2]Department of Human Physiology, College of Medicine and Health Science, Abia State University, Uturu.
[3]Department of Medical Physiology, School of Medicine and Pharmacy, College of Medicine and Health Science, University of Rwanda.
[4]Department of Physiology, College of Medcine, University of the Niger, Iyienu.
[5]Department of Surgery, Faculty of Medicine and Surgery, Chukwuemeka Odumegwu Ojukwu University Teaching Hospital.
[6]Department of Human Human Biochemistry, Faculty of Basic medical Science, Nnamdi Azikwe Univerisity, Nnewi.
[8]Department of Microbiology, Lagos State University, Lagos State.
[9]Department of Microbiology, Faculty of Biological Sciences, University of Nsukka, Enugu State.

***Corresponding Author: Ifemenam Kosisochukwu Emmanuel**
Department of Physiology, College of Medcine, University of the Niger, Iyienu.
Email ID: Kosisochukwu.ifemenam@uniniger.edu.ng

Article Received on 29/12/2024 **Article Revised on 19/01/2025** **Article Accepted on 09/02/2025**

ABSTRACT

The world is faced with many diseases of public health importance. Malaria for Centuries has pluged the world especially the sub- Saharan Africa. This study explores the antimalarial effects of cymbopogon citratus (lemongrass), ocimum gratissimum, and azadirachta indica on the hematological indices in wista rats. Fifty male mice were purchased from a local market, animals were maintained with normal laboratory chow (Grower feed) and water ad libitium. The animals were acclimatized for two weeks before induction of the Plasmodium berghei and ethanolic leave extract of Azadirachta indica, Cymbopogon citratus and Ocimum gratissimum. Plasmodium berghei were inoculated intraperitoneally with 0.2ml blood suspension. Group A was the Negative control group, induced with plasmodium berghei without any treatment. Group B was Positive control, received only food and water. Group C was induced with P. berghei and treated with (500 mg/kg) of Azadirachta indica. Group D was induced with P.berghei and treated with (100 mg/kg) ethanolic extract of Cymbopogon citratus. Group E was induced with P.berghei and treated with (500mg/kg) ethanolic extract of Cymbopogon citratus. Group F was induced with P.berghei and treated with 100mg/kg) ethanolic extract of Ocimum gratissimum. Group G was induced with P. berghei and treated with (500mng/kg) ethanolic extract of Ocimum gratissimum. Group H was induced with P.berghei and treated with (100mg/kg) ethanolic extract of Azadirachta indica. Group I was induced with P.berghei and treated with standard drug. Group J was induced with P. berghei and treated with (500mg/kg) ethanolic extracts of the leaves (Azadirachta indica, Cymbopogon citratus, and Ocimum gratissimum). The Administration of the extract lasted for 7 days.

There was significant decrease in the body weight in-group A (p=0.01), groups B and D had a significant increase (p=0.02, p=0.02). Groups C, E, F, and H had an insignificant increase in the body weight, and group G and I had an insignificant decrease in the bodyweight when the initial weight was compared to the final weight. There was significant decrease in RBC level in group A compared to B (p=0.00). Groups C, D, E, F, G, H, I and J had a significant increase (p=0.01, p=0.00, p=0.02, p=0.00, p=0.01, p=0.03, p=0.02, p=0.04) compared to group A. The Hemoglobin level showed a significant decrease in group A compared to B (p=0.03). Groups C, D, E, G, H, and J had a significant increase (p=0.02, p=0.02, p=0.01, p=0.01, p=0.02, p=0.03), while group I and F had an insignificant increase (p=0.16, p=0.07) compared to group B. The pack cell volume showed a significant decrease in group A compared to B (p=0.02). Groups C, D, E, F, G, H, I and J (p=0.00, p=0.01, p=0.00, p=0.02, p=0.00, p=0.00, p=0.02, p=0.01) had a significant increase compared to group A. There was

significant decrease in MCV level in group A compared to B (p=0.01). Groups C, D, E, F, G, H, I and J had a significant increase (p=0.01, p=0.01, p=0.02, p=0.00, p=0.01, p=0.03, p=0.02, p=0.04) compared to group A. The MCH result demonstrated a significant decrease in group A compared to B (p=0.02). Groups C, D, E, F, G, H, I and J had a significant increase (p=0.02, p=0.01, p=0.02, p=0.01, p=0.01, p=0.02, p=0.03, p=0.04) compared to group A. The MCHC result showed a significant decrease in group A compared to B (p=0.02). Groups C, D, E, F, G, H, I and J had a significant increase (p=0.01, p=0.01, p=0.00, p=0.01, p=0.01, p=0.02, p=0.01, p=0.02) compared to group A. insignificant decrease in WBC level in group A compared to B (p=0.13). Groups C and F had an insignificant increase (p=0.21, p=0.07), while groups D, E, G, H, I and J had a significant increase (p=0.01, p=0.01, p=0.00, p=0.02, p=0.03, p=0.01) compared to group A. The platelet count result demonstrated an insignificant increase in group A compared to B (p=0.92). Groups D, E, H, and I had an insignificant increase (p=0.06, p=0.36, p=0.36, p=0.87), while groups F and G had an insignificant decrease (p=0.87, p=0.15) and group J had a significant increase (p=0.01) compared to group A. There was a significant decrease in lymphocyte count in group A compared to B (p=0.01). Groups C, D, E, F, G, H, I and J had a significant increase (p=0.00, p=0.02, p=0.01, p=0.01, p=0.01, p=0.03, p=0.01, 0.03) compared to group A. The monocyte count result showed a significant decrease in group A when compared to B (p=0.04). Groups C, E, F, G, H, I, and J (p=0.03, p=0.01, p=0.04, p=0.02, p=0.02, p=0.05) had a significant increase while group D had an insignificant (p=0.23) increase compared to group A. Granulocyte count result revealed a significant decrease in group A compared to B (p=0.00). Groups C, D, E, F, G, H, I and J had a significant increase (p=0.01, p=0.00, p=0.01, p=0.01, p=0.01, p=0.01, p=0.01, p=0.01) compared to group A. Cymbopogon citratus, Ocimum gratissimum, and Azadirachta indica showed improved levels of red blood cells, haemoglobin, pack cell volume, MCV, MCH, and MCHC, while WBC improvement was dose and extract dependent; thus, platelet count had no effects following the administration of these extracts. Differential white blood cell count was improved following the administration of Cymbopogon citratus, Ocimum gratissimum, and Azadirachta indica. Further, it showed that malaria patients had decreased weight gain following an infestation by P. berghei. Treatments with Cymbopogon citratus improved weight gain, while A. indica and Ocimum gratissimum had no impact on weight gain.

INTRODUCTION

Malaria is a major public health threat, especially in tropical and subtropical countries. Plasmodium species, transmitted through the bite of an infected female Anopheles mosquito, cause it(Tajbakhsh *et al.*, 2021). *P. falciparum* is the most virulent, responsible for the highest morbidity and mortality. It is also the predominant species in sub-Saharan Africa, with the highest number of malaria cases and deaths in the world (Tajbakhsh *et al.*, 2021). Malaria remains one of the worst perils to tropical countries globally. It is a killer and debilitating disease affecting the physical and economic well-being of individuals living in endemic areas of Africa (Ukpai and Amaechi, 2012). Plasmodium parasite infected malaria is the leading poverty related disease that weakens a country's development. It is endemic in tropical and sub-tropical regions including parts of Africa, Asia, and the Americas (Bess-Bila *et al.*, 2021a). *P. falciparum* is the most lethal form of malaria, causing high-spiking fevers, chills, malaise, headaches, and myalgias, as well as gastrointestinal symptoms. After infection, the stages of malaria in the human body include the hepatic and erythrocyte stages (Snow, 2015). Herbal medicines have advantages over modern medicines, including fewer side effects, cost-effectiveness, and affordability encouraging the herbal-based drug discovery. Several naturally occurring, semisynthetic, and synthetic antimalarial medications are on the market (Mohammadi *et al.*, 2020). Malaria has been treated using traditional herbal remedies for thousands of years. The first antimalarial drug in the West was quinine, derived from the bark of the Cinchona plant. In 1632, bark infusions were used. Quinine's therapeutic properties were crucial in the development of synthetic medications, marking a turning point in malaria treatment history (Adebayo and Krettli, 2011; Habluetzel *et al.*, 2019). *Azadirachta indica*, a medicinal plant known for its neem tree properties, is used in traditional medicine systems worldwide for malaria prevention and treatment. Its antipyretic properties, found in leaves, stem bark, roots, and fruits, may contribute to its efficacy in managing malaria fevers. In vitro and in vivo experiments have shown that the plant contains molecules that interfere with fever pathophysiology, the inflammatory response, and humoral and cell-mediated immunity regulation (Habluetzel *et al.*, 2019). Despite its antimalaria effect, the leaf extract of *A. indica* shows analgesic, anthelminthic, antibacterial, antifungal, antihyperglycemic, anti-inflammatory, antiviral, antimalarial, antipyretic, insecticidal, hypercholesteremic, and hypoglycemic activities in an experimental model (Achi *et al.*, 2018). Traditional medicine has used *Cymbopogon citratus* (lemon grass) as an herbal infusion to treat fever and malaria. Lemon grass is well-known for its culinary applications, which add a distinct citrusy flavour to a variety of dishes and beverages. Furthermore, recent research has revealed that lemon grass has antimicrobial and anti-inflammatory properties, making it a promising candidate for the development of new therapeutic agents. Whole plant extracts, on average, have higher biological activity than purified compounds (Chukwuocha *et al.*, 2016a). *O. gratissimum* belong to the family Lamiaceae and its health benefits are associated with *Ocimum* essential oils and revealed that the anti-viral, anti-microbial, antioxidant, and anti-cancer properties of the plants. Also, the oils have been linked to anti-malaria activities

as indicated by David-Ileke and Adesina, (2019). Malaria can cause anemia and other hematological changes that can be life-threatening, as recurrent incidents can lead to life-threatening anemia and metabolic acidosis (Akinosoglou *et al.,* 2012; Al-Salahy *et al.,* 2016; White, 2018).

Haematological indices involve three major cellular components of blood are red blood cells (erythrocytes), white blood cells (leukocytes) and platelets (thrombocytes) (Bolliger and Everds, 2012), and consist of immunocytes (T and B cells) and their diseases states (Moreno and Wiegand, 2014). However, blood plays a role in transporting and delivering oxygen. Other essential substances and nutrients to every organ remove waste products of metabolism and defend the body against infections via the inflammatory response . Haematological parameters assessment concerning the explanation of blood-related functions of a plant extract; could act as a pathological reflector and as an indicator of the physiological state of an animal (Jorum and Piero, 2016). Also, malaria has been linked to abnormalities of hematological indices, including those related to red blood cells (RBCs), white blood cells (WBCs) and platelets (Kotepui *et al.,* 2014), thus results in anaemia. Plasmodium paraistes is linked to leukaemia, anaemia, and thrombocytopenia, which are specific to malaria diagnosis and play a crucial role in the development of these haematological disorders (Omarine Nlinwe and Nange, 2020). Report has shown that malaria parasite results in anaemia linked to low levels of mean corpuscular volume, mean concentration of haemoglobin, and mean corpuscular haemoglobin concentration, with a high levels of thrombocytopenia and neutropenia (Elkhalifa *et al.,* 2021).

Herbal medicines have advantages over modern medicines, including fewer side effects, cost-effectiveness, and affordability encouraging the herbal-based drug discovery. Several naturally occurring, semisynthetic, and synthetic antimalarial medications are on the market (Mohammadi *et al.,* 2020). Malaria has been treated using traditional herbal remedies for thousands of years. The first antimalarial drug in the West was quinine, derived from the bark of the Cinchona plant. In 1632, bark infusions were used. Quinine's therapeutic properties were crucial in the development of synthetic medications, marking a turning point in malaria treatment history (Adebayo and Krettli, 2011; Habluetzel *et al.,* 2019).

Azadirachta indica is an indigenous plant to India with economic and medicinal value. Humanity has gained its medicinal value to treat ailments of several kinds. Neem is a member of the mahogany family, Meliaceae (Kumar and Navaratnam, 2013). *Azadirachta indica* is called the "Divine tree" which is attributed to its diverse medicinal values to humanity because of its secondary metabolites (Islas *et al.,* 2020). Neem is known for cold pressed seed oil used as insecticides, cosmetic, medicinal, and agricultural effects. However, other pharmacological effects by Neem are malaria fevers, diabetes, heartburn, gastrointestinal disorders, E.t.c (Sujarwo *et al.,* 2016). Neem is considered as the bitter gem and most valued trees with highly significant medicinal activities, and is the most promising plant of numerous pharmacological activities in the 21st century (Maji and Modak, 2021).

Cymbopogon citratus (Lemongrass) is a genus of about 55 species that are indigenous in tropical and semi-tropical areas of Asia and are cultivated in South and Central America, Africa and other tropical countries. These are tufted perennial C_4 grasses with numerous stiff stems arising from a short, rhizomatous rootstock (Weiss 1997; Kumar *et al.,* 2000), as with citrus flavour, and can be dried and powdered or used fresh. *Cymbopogon citratus, Stapf* (Lemongrass) is used in teas, soups, and curries, and is suitable for poultry, fish, and seafood. Cymbopogon originated from the Greek word "kymbe - pogon" meaning boat-beard (due to its flower spike configuration) and *citratus* (Latin) means lemon-scented leaves (Shah *et al.,* 2011).

Ocimum gratissimum (OG) is a perennial herb belonging to the lamiaceae family. *Ocimum gratissimum* has origin from Asia and Africa. In Nigeria and other parts of the world, it is used as a traditional vegetable c ondiment and oral care products. Furthermore, OG had been shown to possess numerous pharmacological properties hence its use in traditional or alternative medicine (Ojo *et al.,*2019). It is a medicinal plant widely grown in tropical and subtropical regions with the leaf decoction usually taken in folk medicine to enhance erectile performance in men although the probable mechanism of actions remains undetermined (Ojo *et al.,*2019). *Ocimum gratissimum* L is commonly known as clove basil or lemon basil, a polymorphic branched, aromatic shrub and a culinary herb with broad applications (Pandey, 2017). Ocimum comprises about 65 species, most native to Africa (**Flora Mesoamericana, 2018**). However, many Ocimum species have been cultivated for thousands of years, particularly across the Mediterranean Basin and southern Asian regions (Bhasin, 2012). As a result, it has numerous variable with distinct polymorphic species treated as different species and subspecies.

MATERIALS AND METHODS

Ethical approval

Ethical approval was obtained from the Animal ethics committee, Abia State University, Uturu.

Plant collection

Samples of *Azadirachta indica, Cymbopogon citratus* and *Ocimum gratissimum* were harvested from a farm at Okofia Community, Otolo in Nnewi, Anambra state. The botanical identification and authentication were confirmed in the herbarium of Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State.

Plant extraction

Azadirachta indica, *Cymbopogon citratus* and *Ocimum gratissimum* leaves were washed in running tap water to remove dirt and air-dried under ambient temperature. The dried leaves were milled into a coarsely powdered form using a local blender. Two hundred and fifty grams of the dried leaves of *Azadirachta indica*, *Cymbopogon citratus* and *Ocimum gratissimum* were macerated in 1000 ml of 95 % Absolute Ethanol(BDH England) for 48 hours. It was filtered using a porcelain cloth and was further filtered using Whatman No 1 filter paper into a clean glass beaker. The filtrate was concentrated using a Rotatory Evaporator (TT-55 Techmel&Techmel, USA) and dried further using a Thermostat Oven (DHG 9021A PEC Medicals, USA) at 45 ^{O}C into a gel-like form. The extracts were preserved in airtight container and kept in a refrigerator for further usage. The extraction method was done with modifications as described according to the method employed by Al-Attar and Abu-Zeid, (2013).

Experimental design

Fifty male mice weighing 21-35g were obtained from the Animal House, Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Abia State University, Uturu. Animals were kept in standard cages at a room temperature of 27±2 °C. The animals were maintained with normal laboratory chow (Grower feed) and water ad libitium. The animals were acclimatized for two weeks before induction of the Plasmodium berghei and ethanolic leave extract of *Azadirachta indica*, *Cymbopogon citratus* and *Ocimum gratissimum*. The animals were kept on 12hours light and dark cycles.

Plasmodium berghei ANKA strain parasitized erythrocytes was obtained from donor mice (Department of Zoology, University of Nigeria, Nsukka). Blood was collected via ocular puncture and diluted in 1:20 of 0.9% normal saline. The mice were inoculated intraperitoneally with 0.2ml blood suspension (Basir *et al.,* 2012a). The animals were observed for four days without treatment, after which parasite level was estimated quantitatively as described by the method of Fidock *et al.,* (2004). The tail of the mice was punctured to collect small drops of blood, which was used to make a thin smear on a slide. The smears were allowed to dry and fixed with methanol and stained with 10 % Leishman stain on the side containing the smear for a period of 5-10 minutes after which it was rinsed with distilled water and allow to air dry. Immersion oil was dropped on the slide to increase its refractive index, and the slide were viewed under a microscope with a ×100 magnification field *(Fidock et al.,* 2004; Okokon *et al.,* 2022).

Group A was the Negative control group, induced with plasmodium berghei without any treatment.

Group B was Positive control, received only food and water

Group C was induced with P. berghei and treated with (500 mg/kg) of *Azadirachta indica.*

Group D was induced with P.berghei and treated with (100 mg/kg) ethanolic extract of *Cymbopogon citratus.*

Group E was induced with P.berghei and treated with (500mg/kg) ethanolic extract of *Cymbopogon citratus*

Group F was induced with P.berghei and treated with 100mg/kg) ethanolic extract of *Ocimum gratissimum*

Group G was induced with P. berghei and treated with (500mng/kg) ethanolic extract of *Ocimum gratissimum*.

Group H was induced with P.berghei and treated with (100mg/kg) ethanolic extract of *Azadirachta indica*.

Group I was induced with P.berghei and treated with standard drug.

Group J was induced with P. berghei and treated with (500mg/kg) ethanolic extracts of the leaves (*Azadirachta indica*, *Cymbopogon citratus*, and *Ocimum gratissimum*).

The Administration of the extract lasted for 7 days**.**

Acute toxicity of plant extracts

The median lethal dose (LD50) of the ethanoic ethanol leaf *Azadirachta indica*, *Cymbopogon citratus and Ocimum gratissimum* were determined using Lorkes method (Lorke, 1983), and it was divided into two phases. This was conducted in the Department of Physiology, Faculty of Basic Medical Sciences, Abia State University, Uturu.

Sample collection

At the end of the experiment following the administration of ethanolic leaf extract of *Azadirachta indica*, *Cymbopogon citratus* and *Ocimum gratissimum*, the mice were anesthetized using chloroform in an enclosed container for 2-minutes. Blood was collected from the retro-orbital sinus using heparinized capillary tube as described by Parasuraman et al. (2010), and put in EDTA tube, which was allowed to cool for 5-minutes. Blood put in the EDTA container was assayed for hematological indices using Automated blood analyzer Sysmex XT-2000i (Sysmex, Kobe, Japan).

Determination of hematological indices

Hematological parameter (Red blood cell, Pack cell volume, and white blood cell) was done using an automated blood analyzer Sysmex XT-2000i as described by the method of Gheith and El-Mahmoudy (2018).

Statistical analysis

Data obtained from this study was analysed using Statistical Package for Social Sciences (SPSS) version 25 (IBM, USA, 2018). Data obtained for haematological indices, kidney function test, liver enzymes, parasitaemia count, relative kidney, and liver weight was analysed using Analysis of variance (ANOVA) followed by post hoc LSD multiple comparisons. Body weight was analysed using T-dependent test. Data was considered significant at $p<0.05$.

RESULTS

Table 1: Effect of Ethanolic Extracts of *Cymbopogon citratus*, *Occimum gratissimum*, and *Azadirachta indica* on Body Weight Following Plasmodium berghei Induced Toxicity.

	Initial weight (g)	Final weight (g)	P-value	T-value
	MEAN±SEM	MEAN±SEM		
Group A (Malaria only)	28.35±1.37	20.54±0.65	0.01[a]	5.04
Group B (Normal control)	27.56±2.85	38.51±0.40	0.02[a]	-3.41
Group C (Malaria + 500mg/kg EAI)	21.87±2.41	22.43±0.60	0.87[b]	-0.19
Group D (Malaria + 100mg/kg ECC)	20.13±1.94	28.75±3.11	0.02[a]	-5.07
Group E (Malaria + 500mg/kg ECC)	22.73±1.20	28.56±1.48	0.11[b]	-2.73
Group F (malaria + 100mg/kg EOG)	21.00±0.81	23.86±3.09	0.37[b]	-1.16
Group G (malaria + 500mg/kg EOG)	25.20±1.22	25.00±1.50	0.95[b]	0.08
Group H (Malaria + 100mg/kg EAI)	24.70±0.87	25.02±1.21	0.52[b]	-0.73
Group I (Malaria + Standard drug)	26.70±2.21	24.78±0.81	0.30[b]	1.39
Group J (Malaria + 500mg/kg EOG + EAI + ECC)	28.80±0.60	28.97±1.25	0.82[b]	-0.25

Data was analyzed using T-test, and values considered significant at $p<0.05$. SEM: Standard error of mean. BWC: Bodyweight change, EOG: ethanolic leaf extract of *Ocimum gratissimum*, EAI: ethanolic leaf extract of *Azadirachta indica*, ECC: ethanolic leaf extract of *Cymbopogon citratus* ([a]= significant, [b]= not significant)

Table 1 result revealed a significant decrease in the body weight in-group A (*p=0.01*), groups B and D had a significant increase (*p=0.02, p=0.02*). Groups C, E, F, and H had an insignificant increase in the body weight, and group G and I had an insignificant decrease in the bodyweight when the initial weight was compared to the final weight.

Table 2: Effect of Ethanolic Extract of *Cymbopogon Citratus*, *Occimum Gratissimum*, And *Azadirachta Indica* On Red Blood Cells, Hemoglobin, And Pack Cell Volume Following *Plasmodium Berghei* Induced Toxicity.

	Red blood cell (x10^12/l)	Hemoglobin (g/dl)	Pack cell volume (%)
	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group A (Malaria only)	4.73±0.19	8.50±0.74	36.10±0.85
Group B (Normal control)	7.38±0.20[a]	11.60±0.20[a]	43.83±0.64[a]
Group C (Malaria + 500mg/kg EAI)	9.05±0.21[a]	13.43±0.61[a]	53.23±1.48[a]
Group D (Malaria + 100mg/kg ECC)	8.42±0.55[a]	11.87±0.87[a]	48.19±2.72[a]
Group E (Malaria + 500mg/kg ECC)	8.65±0.55[a]	12.63±0.65[a]	50.10±0.52[a]
Group F (malaria + 100mg/kg EOG)	7.03±1.06[a]	10.48±1.97[a]	43.39±4.92[a]
Group G (malaria + 500mg/kg EOG)	8.48±0.75[a]	12.50±1.31[a]	49.40±2.99[a]
Group H (Malaria + 100mg/kg EAI)	9.09±0.24[a]	13.45±1.16[a]	50.73±0.29[a]
Group I (Malaria + Standard drug)	7.63±0.24[a]	11.13±0.35[b]	43.33±0.56[a]
Group J (Malaria + 500mg/kg EOG + EAI + ECC)	8.29±0.27[a]	13.10±0.10[a]	49.30±0.21[a]
F-value	6.39	2.54	5.71

Data was analyzed using ANOVA, and values considered significant at $p<0.05$. SEM: Standard error of mean. EOG: Ethanolic leaf extract of *Ocimum gratissimum*, EAI: Ethanolic leaf extract of *Azadirachta indica*, ECC: Ethanolic leaf extract of *Cymbopogon citratus* ([a]= significant, [b]= not significant).

Table 2 result revealed a significant decrease in RBC level in group A compared to B (*p=0.00*). Groups C, D, E, F, G, H, I and J had a significant increase (*p=0.01, p=0.00, p=0.02, p=0.00, p=0.01, p=0.03, p=0.02, p=0.04*) compared to group A. The Hemoglobin level showed a significant decrease in group A compared to B (*p=0.03*). Groups C, D, E, G, H, and J had a significant increase (*p=0.02, p=0.02, p=0.01, p=0.01, p=0.02, p=0.03*), while group I and F had an insignificant increase (*p=0.16, p=0.07*) compared to group B. The pack cell volume showed a significant decrease in group A compared to B (*p=0.02*). Groups C, D, E, F, G, H, I and J (*p=0.00, p=0.01, p=0.00, p=0.02, p=0.00, p=0.00, p=0.02, p=0.01*) had a significant increase compared to group A.

Table 3: effect of ethanolic extract of *Cymbopogon citratus*, *Occimum gratissimum* and *Azadirachta indica* on MCV, MCH and MCHC following *plasmodium berghei* induced toxicity.

	MCV(FL)	MCH (pg)	MCHC (g/dl)
	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group A (Malaria only)	39.33±3.28	10.42±0.43	203.33±15.03
Group B (Normal control)	58.20±0.25 [a]	15.63±0.03[a]	266.33±0.67[a]
Group C (Malaria + 500mg/kg EAI)	58.93±0.49 [a]	15.33±0.07[a]	257.33±1.20[a]
Group D (Malaria + 100mg/kg ECC)	52.43±2.01 [a]	14.27±0.09[a]	262.33±5.24[a]
Group E (Malaria + 500mg/kg ECC)	54.80±0.67 [a]	14.33±0.07[a]	260.00±3.51[a]
Group F (malaria + 100mg/kg EOG)	58.97±1.87[a]	14.53±0.64[a]	254.67±16.75[a]
Group G (malaria + 500mg/kg EOG)	55.20±0.59[a]	14.70±0.26[a]	261.67±8.35[a]
Group H (Malaria + 100mg/kg EAI)	54.80±1.04[a]	16.00±0.21[a]	282.00±9.29[a]
Group I (Malaria + Standard drug)	55.03±2.05[a]	14.77±0.65[a]	269.33±3.18[a]
Group J (Malaria + 500mg/kg EOG + EAI + ECC)	57.83±2.08[a]	15.60±0.32[a]	266.30±4.28[a]
F-value	11.55	19.77	5.97

Data was analyzed using ANOVA, and values considered significant at $p<0.05$. SEM: Standard error of mean. EOG: Ethanolic leaf extract of *Ocimum gratissimum*, EAI: Ethanolic leaf extract of *Azadirachta indica*, ECC: Ethanolic leaf extract of *Cymbopogon citratus* ([a]= significant, [b]= not significant)

Table 3 shows a significant decrease in MCV level in group A compared to B (*p=0.01*). Groups C, D, E, F, G, H, I and J had a significant increase (*p=0.01, p=0.01, p=0.02, p=0.00, p=0.01, p=0.03, p=0.02, p=0.04*) compared to group A. The MCH result demonstrated a significant decrease in group A compared to B (*p=0.02*). Groups C, D, E, F, G, H, I and J had a significant increase (*p=0.02, p=0.01, p=0.02, p=0.01, p=0.01, p=0.02, p=0.03, p=0.04*) compared to group A. The MCHC result showed a significant decrease in group A compared to B (*p=0.02*). Groups C, D, E, F, G, H, I and J had a significant increase (*p=0.01, p=0.01, p=0.00, p=0.01, p=0.01, p=0.02, p=0.01, p=0.02*) compared to group A.

Table 4: Effect of Ethanolic Extract of *Cymbopogon Citratus*, *Ocimum Gratissimum*, and *Azadirachta Indica* on White Blood Cell And Platelet Count Following *Plasmodium Berghei* induced toxicity.

	White blood cell (x10^9/l)	Platelet count (10^9/L)
	MEAN±SEM	MEAN±SEM
Group A (Malaria only)	5.30±0.50	642.00±71.59
Group B (Normal control)	7.03±0.067[b]	636.00±23.01[b]
Group C (Malaria + 500mg/kg (EAI)	6.7±0.33[b]	500.00±26.29[a]
Group D (Malaria + 100mg/kg (ECC)	8.90±1.80[a]	763.67±2.60[b]
Group E (Malaria + 500mg/kg (ECC)	8.50±0.58[a]	729.00±31.79[b]
Group F (malaria + 100mg/kg EOG)	7.50±0.87[b]	632.67±46.06[b]
Group G (malaria + 500mg/kg EOG)	11.90±1.12[a]	633.33±32.34[b]
Group H (Malaria + 100mg/kg EAI)	10.93±0.22[a]	696.67±15.10[b]
Group I (Malaria + Standard drug)	11.53±0.54[a]	651.67±75.30[b]
Group J (Malaria + 500mg/kg EOG + EAI + ECC)	9.00±0.00[a]	889.00±28.29[a]
F-value	7.56	6.08

Data was analyzed using ANOVA, and values considered significant at $p<0.05$. SEM: Standard error of mean. EOG: Ethanolic leaf extract of *Ocimum gratissimum*, EAI: Ethanolic leaf extract of *Azadirachta indica*, ECC: Ethanolic leaf extract of *Cymbopogon citratus*([a]= significant, [b]= not significant)

Table 4 result revealed an insignificant decrease in WBC level in group A compared to B (*p=0.13*). Groups C and F had an insignificant increase (*p=0.21, p=0.07*), while groups D, E, G, H, I and J had a significant increase (*p=0.01, p=0.01, p=0.00, p=0.02, p=0.03, p=0.01*) compared to group A. The platelet count result demonstrated an insignificant increase in group A compared to B (*p=0.92*). Groups D, E, H, and I had an insignificant increase (*p=0.06, p=0.36, p=0.36, p=0.87*), while groups F and G had an insignificant decrease (*p=0.87, p=0.15*) and group J had a significant increase (*p=0.01*) compared to group A.

Table 5: Effect of Ethanolic Extract of *Cymbopogon Citratus*, *Occimum Gratissimum*, And *Azadirachta Indica* On Differential White Blood Cell Count Following *Plasmodium Berghei* Induced Toxicity.

	Lymphocytes (%)	Monocytes (%)	Granulocyte (%)
	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group A (Malaria only)	34.90±5.78	1.40±0.35	1.80±0.23
Group B (Normal control)	89.27±0.42 [a]	4.63±0.03[a]	6.57±0.07[a]
Group C (Malaria + 500mg/kg EAI)	89.57±2.27 [a]	4.70±1.10[a]	4.33±0.88[a]
Group D (Malaria + 100mg/kg ECC)	83.03±8.89 [a]	2.63±0.23[b]	5.90±0.28[a]
Group E (Malaria + 500mg/kg ECC)	86.43±4.24 [a]	4.43±0.33[a]	4.73±0.52[a]
Group F (malaria + 100mg/kg EOG)	71.80±13.59[a]	4.1±0.83[a]	5.63±1.04[a]
Group G (malaria + 500mg/kg EOG)	86.17±4.39[a]	4.60±0.44[a]	4.80±0.12[a]
Group H (Malaria + 100mg/kg EAI)	79.87±12.23[a]	4.03±0.61[a]	4.40±0.058[a]
Group I (Malaria + Standard drug)	75.77±15.38[a]	3.90±1.10[a]	4.70±0.70[a]
Group J (Malaria + 500mg/kg EOG + EAI + ECC)	79.32±11.92[a]	3.47±0.97[a]	4.80±0.00[a]
F-value	2.98	2.26	3.63

Data was analyzed using ANOVA, and values considered significant at $p<0.05$. SEM: Standard error of mean. EOG: Ethanolic leaf extract of *Ocimum gratissimum*, EAI: Ethanolic leaf extract of *Azadirachta indica*, ECC: Ethanolic leaf extract of *Cymbopogon citratus* ([a]= significant, [b]= not significant).

Table 5 result demonstrated a significant decrease in lymphocyte count in group A compared to B (*p=0.01*). Groups C, D, E, F, G, H, I and J had a significant increase (*p=0.00, p=0.02, p=0.01, p=0.01, p=0.01, p=0.03, p=0.01, 0.03*) compared to group A. The monocyte count result showed a significant decrease in group A when compared to B (*p=0.04*). Groups C, E, F, G, H, I, and J (*p=0.03, p=0.01, p=0.04, p=0.02, p=0.02, p=0.05*) had a significant increase while group D had an insignificant (*p=0.23*) increase compared to group A. Granulocyte count result revealed a significant decrease in group A compared to B (*p=0.00*). Groups C, D, E, F, G, H, I and J had a significant increase (*p=0.01, p=0.00, p=0.01, p=0.01, p=0.01, p=0.01, p=0.01, p=0.01*) compared to group A.

DISCUSSION

Medicinal plant has gained ground in the treatment and management of malaria infection caused by plasmodium species of different mosquitoes (Mohammadi *et al.*, 2020; Rudrapal and Chetia, 2021). Thus, malaria is an important life-threatening infection in numerous tropical countries around the globe with an increase in drug resistance in recent times, which potentiates the need to contribute to malaria reduction in the future (*Arome et al.*, 2016).

The study investigates the effect of ethanolic extract of *Azadirachta indica*, *Cymbopogon citratus* and *Ocimum gratissimum* on the hematological indices, following Plasmodium berghei-induced malaria in mice.

The study findings showed that *Plasmodium berghei* had a significant decrease in the body weight, while treatments with *Azadirachta indica* and *Ocimum gratissimum* at low and high dose had no effect on body weight. Thus, *Cymbopogon citratus* had improved weight gained at low dose, and at high dose, there was no impact. The mechanism following significant decline in the body weight is attributed to the presence of suppressive impact of the plasmodium on the satiety centers, which results to decrease in food and water intake as well as decrease in metabolic rate. The decrease in body weight may also be the consequence of disturbed metabolic function and hypoglycemia, which have been reported to be associated with malaria infection (Basir *et al.*, 2012b). The study corresponds to the findings of Toma et al. (2015), which indicated a significant weight loss following *P. berghei* infested mice. Also, Basir et al. (2012b) reported a significantly decrease in the body weight following *P. berghei* infested mice, which corroborates the study. Sowunmi et al. (2007) showed a significant weight loss following *Plasmodium falciparum* induced malaria, which agree with the study findings. Aghahowa and Okolocha, (2018), Omoirri et al. (2020), Baah et al. (2020), and Syukriah et al. (2022) reported a significant weight in loss in the plasmodium infested mice following *P. berghei* induction, which agrees with the study findings. However, treatments with *C. citratus* at low dosage demonstrated a significant reduction in the body weight, following *P. berghei* infested mice. The significant decline in the body weight could be attributed to the presence of alkaloids, which has the potency of inhibiting the satiety centers in the hypothalamus. Further, it was documented that Groups C, E, F, and H had an insignificant increase in the body weight, and group G and I had an insignificant decrease in the bodyweight, with the physiology not well understood.

Malaria is a pathogenic illness that primarily destroys red blood cells in mammals (RBCs). The morphology and rheological characteristics of malaria-infected RBCs change, and the changed rheological properties of RBCs have a substantial influence on disease pathogenesis (Kwon *et al.*, 2019). Haemoglobin (HGB) is an intracellular protein found in the red blood cell, and its value often decreases in malaria patients because growing parasites consume haemoglobin. This decrease

in HGB levels can lead to anaemia, a common malaria complication. Anaemia can cause fatigue, weakness, and shortness of breath, and may require blood transfusions in severe cases (Asangha *et al.*, 2017a). The study demonstrated a significant decrease in the red blood cells, PCV, and hemoglobin level following *P. berghei*, which is attributed to cytotoxic nature of plasmodium parasites in the liver, and causes the hemoglobin levels, red blood cells, and PCV levels decrease. The study agrees with the findings of Bess Billa et al. (2021b) reporting a significant decline in the RBC, PCV, and Hb levels in plasmodium berghei infested malaria. Kabiru et al. (2016), Elkhalifa et al. (2021), Asangha et al. (2017a), Akuodor et al. (2023) showed that *P. berghei* indicated a significant decline in PCV, RBC, and Hb levels following malaria infested mice, which corroborates the study outcome. Furthermore, Wang et al. (2021) reported a significant increase in RBC and PCV levels, which refutes the study outcome. Also, Kwon et al. (2019), Karimi et al. (2014) had inconsistency to the study findings revealing a significant increase in the RBC activity following malaria parasite infection. However, report of El-Assaad et al. (2013) reported a significant increase in the RBC level in infected *P. berghei* activity, which disagrees to the study outcome. Shittu et al. (2016b), Osonwa et al. (2017) reported a significant rise in PCV levels following *P. berghei* infected mice, which disagree the study's report. Ojueromi et al. (2022) reported a significant reduction in the Hb and RBC levels of *P. berghei* infested mice, which is in accordance with the study. However, treatments with the different leaves extract and standard drug showed ameliorative effect by increasing the levels of RBC, PCV, and Hb levels in groups C to J when compared to A. The physiology following the significant decrease in these levels of RBC, PCV, and Hb is attributed to flavonoids, alkaloids, and polyphenols in the leaves of *Azadirachta indica*, *Cymbopogon citratus* and *Ocimum gratissimum*. The study Shittu et al. (2016b), Osonwa et a. [(2017)] following *O. gratissimum* treatments, which had significant decrease in the PCV levels contradicting the study findings. Also, the report Bess-Bila *et al.*,(2021b) and Samuel et al. (2021) had similarities to this study revealing a significant increase in PCV and RBC levels following treatment with *C. citratus* against *P. berghei* activity. Akin-Osanaiye et al. (2015) reported a significant increase in the PCV levels of infested *P. berghei* mice following *A. indica* treatments, which corresponds to the study findings. However, Artemether showed a significant increase in the PCV levels in *P. berghei* induced malaria, which corroborates the study outcome. Achi et al. (2018) reported a significant increase in RBC, PCV, and Hb following *A. indica* and Artemether-lumartem, which is in line with the study report. Treatments with Artemether showed significant increase RBC, PCV, and Hb level in *P. berghei* infested mice, which agrees with the report of Adikwu and Ajeka (2022) reporting a significant increase in RBC, PCV, and Hb level in *P. berghei* infested mice following administration of Artemether.

Anaemia is a clinical condition that is characterized by low levels of haemoglobin, however, MCV, MCH, and MCHC levels are linked to different anaemic conditions such as microcytic or macrocytic anaemia (Maner and Moosavi, 2022). The study findings indicated a significant decrease in the MCV, MCH, and MCHC levels in plasmodium infested mice compared to the normal control. The mechanism of action following plasmodium infestation has been linked to reduction in the MCV, MCH, and MCHC levels, which results from either intravascular or extravascular hemolysis because of the schizonticide activity (Haldar and Mohandas, 2009). Kotepui et al. (2014, 2015) reported a significant higher levels of MCV, MCH, and MCHC following *P. falciparum*, which contradict the study outcome. However, Saganuwan and Onyeyili, (2012) reported a significant reduction in the MCV levels in plasmodium infested mice, which agrees with the study findings and contradicts the study outcome following a significant increase in the MCH and MCHC levels following *P. berghei* infestation. Asangha et al. (2017a) reported a significant decrease in the MCHC levels of plasmodium infestation in mice, which agrees with the study outcome, and disagree with the study findings revealing a non-significant differences in the MCV and MCH activity following *P. berghei* infestation. Godwin et al. (2011) showed a significant reduction in the MCV levels following *P. berghei* infestation, which corroborates the study findings. Treatments showed that the ethanolic leaf extracts of *Azadirachta indica*, *Cymbopogon citratus* and *Ocimum gratissimum* indicated a significant increase in the MCV, MCH, and MCHC levels from groups C to J when compared to A. The rationale following the physiological increase in these parameters could be linked to the phytochemicals such as alkaloids, flavonoids, tannins, and saponins present. These phytonutrients tend to combat the plasmodium activities that causes changes in the shape and numbers of red blood cell indices, thus, helps in the buildup of red blood cells indices. Bess Bila *et al.* (2021) reported a significant increase in the MCV, MCH, and MCH levels following treatments with *Cymbopogon citratus* in *P. berghei* infested mice, which is in line with study findings. Samuel et al. (2021) reported a significant increase in the MCH, MCV, and MCHC levels following *Cymbopogon citratus* in *P. berghei* infested mice, which is in line with this study.

The study showed that a non-significant decrease in WBC level in group A compared to B. Groups C and F had an insignificant increase, while groups D, E, G, H, I and J had a significant increase compared to group A. The non-significant difference shown by WBC and platelet count is not well understood. The report of Bess-Bila *et al.*,(2021b), Asangha et al. (2017b), Oluyemi and Folayele, (2018), Momoh et al. (2015) had a significant increase in WBC level in Plasmodium berghei infested mic model, which disagree with the study findings. Kotepui et al. (2014) reported a significant decrease in the WBC levels in plasmodium infested individuals,

which contradicts the study report. Also, the findings of Asangha et al. (2017) revealed a significant increase in WBC the *P. berghei* infested mice, which contradicts the study report. Shija et al. (2020) reported a significant increase in WBC level following administration of lemon grass decoction in *P. berghei* infested mice, which disagree with the study findings. Also, report from Bess-Billa et al. (2021) demonstrated a significant decrease in the WBC levels following *C. citratus* in *P. berghei* infested mice, which disagrees with the study findings. Adikwu and Ajeka (2021) reported a significant increase in WBC compared to the *P. berghei* infested mice, which contradicts the study outcome following Artemether administration.

Malaria is known to cause life threatening levels of thrombocytopenia, which is believe to Abs targeting platelets for destruction by the reticuloendothelial system (Gramaglia *et al.*, 2005). However, reports have shown that platelets can interact with parasitized erythrocytes, leukocytes, and endothelium, leading to micro-vessel obstructions and the release of inflammatory mediators (De Azevedo-Quintanilha *et al.*, 2020). This can result in a wide range of clinical manifestations, including fever, anaemia, organ dysfunction, and even death. The platelet count result demonstrated an insignificant increase in group A compared to B. Groups D, E, H, and I had an insignificant increase while groups F and G had an insignificant decrease and group J had a significant increase compared to group A. The physiology behind the non-significant increase is not well understood. The study disagrees with the report of Asangha et al. (2017), Inyang et al. (1987), Gramaglia et al. (2005), De Azevedo-Quintanilha et al. (2020), Boonyapranai et al. (2022) who revealed a significant decrease in the platelet count following *P. berghei* infested mice. Kotepui et al. (2014) reported a significant decrease in the platelet levels in plasmodium infested individuals, which contradicts the study report. However, treatments with the leaves extract of *Azadirachta indica*, *Cymbopogon citratus* and *Ocimum gratissimum* showed no significant changes in the platelet count. The mechanism of action is not fully elucidated; however, the report of Achi et al. (2018) demonstrated a significant decrease in the platelet count following *A. indica*, which contradict the study findings. Bess-Billa et al. (2021) demonstrated a significant increase in the platelet count levels following *C. citratus* in *P. berghei* infested mice, which disagrees with the study findings. Sadi and Imam, (2019) reported a non-significant change in the white blood cell following the aqueous extracts of *C. citratus* in *P. berghei* infested mice, which agrees with the study findings.

The study demonstrated a significant decrease in lymphocyte count in group A compared to B. Groups C, D, E, F, G, H, I and J had a significant increase compared to group A. The monocyte count result showed a significant decrease in group A when compared to B. Groups C, E, F, G, H, I, and J had a significant increase while group D had an insignificant increase compared to group A. Granulocyte count result revealed a significant decrease in group A compared to B. Groups C, D, E, F, G, H, I and J had a significant increase compared to group A. The physiology responsible for the decrease in the differential white blood cells parameters for malaria infested mice is due to the inflammatory activities of the schizogonic stage of plasmodium parasite, which causes depletion of the immune function. The study agrees with the findings of Asangha et al. (2017), which indicated a significant decline in the lymphocytes, neutrophils, and monocyte count following *P. berghei* infested mice. Bess-Billa et al. (2021) reported a significant decrease lymphocyte count, which is in accordance with the study report; while increase in monocyte count, and granulocytes count showed disagreement to the study report following *P. berghei* infested mice. Bess-Billa et al. (2021) demonstrated a significant decrease in the monocyte levels following *C. citratus* in *P. berghei* infested mice, which disagrees with the study findings. However, lymphocyte count showed a significant increase, which is in line with the study findings.

CONCLUSION

Cymbopogon citratus, *Ocimum gratissimum*, and *Azadirachta indica* showed improved levels of red blood cells, haemoglobin, pack cell volume, MCV, MCH, and MCHC, while WBC improvement was dose and extract dependent; thus, platelet count had no effects following the administration of these extracts. Differential white blood cell count was improved following the administration of *Cymbopogon citratus*, *Ocimum gratissimum*, and *Azadirachta indica*. Further, it showed that malaria patients had decreased weight gain following an infestation by *P. berghei*. Treatments with *Cymbopogon citratus* improved weight gain, while *A. indica* and *Ocimum gratissimum* had no impact on weight gain.

REFERENCE

1. Achi, N. K., Onyeabo, C., Nnate, D. A., Ekeleme-Egedigwe, C. A., Kalu, I. K., Chibundu, I. C., and Wokoma, G. C. Therapeutic effects of Azadirachta indica A.Juss. leaves in malaria-induced male Wistar rats. *Journal of Pharmacy and Pharmacognosy Research*, 2018; *6*(3): 191–204. http://jppres.com/jppres
2. Adebayo, J. O., and Krettli, A. U. Potential antimalarials from Nigerian plants: A review. *Journal of Ethnopharmacology*, 2011; *133*(2): 289–302. https://doi.org/10.1016/J.JEP.2010.11.024
3. Adikwu, E., Ajeka, I. S., and Nworgu, C. O. Amodiaquine-Azithromycin Eradicates Blood and Liver Stages of Plasmodium berghei Infection in Mice. *American Journal of Biomedical Sciences*, 2022; *10*(6): 136–145. https://doi.org/10.5099/aj220300136
4. Akin-Osanaiye, Nok, A. J., Amlabu, E., and Haruna, E. Assessment of Changed in Serum Haematological Parameters in the Assessment of Changed in Serum

Haematological Parameters in the Plasmodium berghei Infected Albino Mice Treated with Neem (Azadirachta indica) Extracts. *International Journal of Chemical and Biomolecular Science*, 2015; *1(3): 148:* 148–152.

5. Akinosoglou, K. S., Solomou, E. E., and Gogos, C. A. Malaria: a haematological disease. *Hematology (Amsterdam, Netherlands)*, 2012; *17*(2): 106–114. https://doi.org/10.1179/102453312X13221316477336
6. Akuodor, G. C., Ezeunala, M. N., Essiet, G. A., John, D. O., Ramalan, M. A., Chilaka, J. U., Ofor, C. C., and Onyebuchi, A. D. Antiplasmodial and hematological indices in Plasmodium berghei infected mice treated with ethanol root extract of Salacia lehmbachii. *Azerbiajan Medical Journal*, 2023; *63*(02): 7577–7587.
7. Al-Attar, A. M., and Abu Zeid, I. M. Effect of tea (camellia sinensis) and olive (olea europaea L.) leaves extracts on male mice exposed to diazinon. *BioMed Research International*, *2013*; 1–6. https://doi.org/10.1155/2013/461415
8. Al-Salahy, M., Shnawa, B., Abed, G., Mandour, A., and Al-Ezzi, A. Parasitaemia and Its Relation to Hematological Parameters and Liver Function among Patients Malaria in Abs, Hajjah, Northwest Yemen. *Interdisciplinary Perspectives on Infectious Diseases*, *2016*; 5954394. https://doi.org/10.1155/2016/5954394
9. Arome, D., Chinedu, E., Ameh, S. F., and Sunday, A. I. Comparative antiplasmodial evaluation of Cymbopogon citratus extracts in plasmodium berghei infected mice. *Journal of Current Research in Scientific Medicine*, 2016; *2*(1): 29–35. https://doi.org/10.4103/2455-3069.184126
10. Asangha, E. E., Igile, G. O., Iwara, I. A., Ebong, P. E., and Eseyin, O. A. Hematological Indices of Plasmodium berghei Infected Mice Treated with Ethanol Extract and Fractions of Nauclea latifolia Roots. *International Journal of Current Microbiology and Applied Sciences*, 2017; *6*(12): 2546–2556. https://doi.org/10.20546/ijcmas.2017.612.295
11. Asangha, E. E., Igile, G. O., Iwara, I. A., Ebong, P. E., and Eseyin, O. A. Hematological Indices of Plasmodium berghei Infected Mice Treated with Ethanol Extract and Fractions of Nauclea latifolia Roots. *Int.J.Curr.Microbiol.App.Sci*, 2017; *6*(12): 2546–2556. https://doi.org/10.20546/ijcmas.2017.612.295
12. Baah, M. K., Mensah, A. Y., Asante-Kwatia, E., Amponsah, I. K., Forkuo, A. D., Harley, B. K., and Adjei, S. In Vivo Antiplasmodial Activity of Different Solvent Extracts of Myrianthus libericus Stem Bark and Its Constituents in Plasmodium berghei -Infected Mice. *Evidence-Based Complementary and Alternative Medicine*, *2020*; 1–10. https://doi.org/10.1155/2020/8703197
13. Basir, R., Rahiman, S. S. F., Hasballah, K., Chong, W. C., Talib, H., Yam, M. F., Jabbarzare, M., Tie, T. H., Othman, F., Moklas, M. A. M., Abdullah, W. O., and Ahmad, Z. Plasmodium berghei ANKA Infection in ICR Mice as a Model of Cerebral Malaria. *Iranian Journal of Parasitology*, 2012; *7*(4): 62–74. /pmc/articles/PMC3537477/
14. Bess Bila, R., Sotoing Taiwe, G., Denis, D., Sofeu, F., Sylvain, O., Bopda, M., Mann, H., Mambou, A. Y., Nji, S., Musa, O., Toukam, L. L., and Kimbi, H. K. Prophylactic antimalarial effects of Cymbopogon citratus (DC.) Stapf (Poaceae) in a mouse model of Plasmodium berghei ANKA infection: normalisation of haematological and serum biochemical status. *GSC Biological and Pharmaceutical Science*, 2021a; *15*(1): 5–17. https://doi.org/10.30574/gscbps.2021.15.1.0084
15. Bess Bila, R., Sotoing Taiwe, G., Denis, D., Sofeu, F., Sylvain, O., Bopda, M., Mann, H., Mambou, A. Y., Nji, S., Musa, O., Toukam, L. L., and Kimbi, H. K. *Prophylactic antimalarial effects of Cymbopogon citratus (DC.) Stapf (Poaceae) in a mouse model of Plasmodium berghei ANKA infection: normalisation of haematological and serum biochemical status*, 2021. https://doi.org/10.30574/gscbps.2021.15.1.0084
16. Bolliger, A. P., and Everds, N. Haematology of the Mouse. *The Laboratory Mouse*, 2012; 331–347. https://doi.org/10.1016/B978-0-12-382008-2.00014-3
17. Boonyapranai, K., Sarakul, O., Somsak, V., and Sukati, S. Effects of Gymnema inodorum Leaf Extract on the Alteration of Blood Coagulation Parameters and Platelet Count in Plasmodium berghei -Infected Mice. *Journal of Parasitology Research*, *2022*; 1–6. https://doi.org/10.1155/2022/4225682
18. Chukwuocha, U. M., Fernández-Rivera, O., and Legorreta-Herrera, M. Exploring the antimalarial potential of whole Cymbopogon citratus plant therapy. *Journal of Ethnopharmacology*, 2016; *193*: 517–523. https://doi.org/10.1016/J.JEP.2016.09.056
19. Chukwuocha, U. M., Fernández-Rivera, O., and Legorreta-Herrera, M. Exploring the antimalarial potential of whole Cymbopogon citratus plant therapy. *Journal of Ethnopharmacology*, 2016; *193*: 517–523. https://doi.org/10.1016/J.JEP.2016.09.056
20. David Ileke, K., and Jacobs, M. A. Toxicity of Ocimum basilicum and Ocimum gratissimum Extracts against Main Malaria Vector, Anopheles gambiae (Diptera: Culicidae) in Nigeria. *Journal of Arthropod-Borne Diseases*, 2019; *13*(4): 362–368. /pmc/articles/PMC7188766/
21. De Azevedo-Quintanilha, I. G., Medeiros-De-Moraes, I. M., Ferreira, A. C., Reis, P. A., Vieira-De-Abreu, A., Campbell, R. A., Weyrich, A. S., Bozza, P. T., Zimmerman, G. A., and Castro-Faria-Neto, H. C. Haem oxygenase protects against thrombocytopaenia and malaria-associated lung injury. *Malaria Journal*, 2020; *19*(1): 1–16. https://doi.org/10.1186/S12936-020-03305-6/FIGURES/6

22. El-Assaad, F., Wheway, J., Mitchell, A. J., Lou, J., Hunt, N. H., Combes, V., and Grau, G. E. R. Cytoadherence of Plasmodium berghei-infected red blood cells to murine brain and lung microvascular endothelial cells in vitro. *Infection and Immunity*, 2013; *81*(11): 3984–3991. https://doi.org/10.1128/IAI.00428-13
23. Elkhalifa, A. M. E., Abdul-Ghani, R., Tamomh, A. G., Eltaher, N. E., Ali, N. Y., Ali, M. M., Bazie, E. A., KhirAlla, A., DfaAlla, F. A., and Alhasan, O. A. M. Hematological indices and abnormalities among patients with uncomplicated falciparum malaria in Kosti city of the White Nile state, Sudan: a comparative study. *BMC Infectious Diseases*, 2021; *21*(1): 1–8. https://doi.org/10.1186/S12879-021-06228-Y/TABLES/4
24. Elkhalifa, A. M. E., Abdul-Ghani, R., Tamomh, A. G., Eltaher, N. E., Ali, N. Y., Ali, M. M., Bazie, E. A., KhirAlla, A., DfaAlla, F. A., and Alhasan, O. A. M. Hematological indices and abnormalities among patients with uncomplicated falciparum malaria in Kosti city of the White Nile state, Sudan: a comparative study. *BMC Infectious Diseases*, 2021; *21*(1): 1–8. https://doi.org/10.1186/S12879-021-06228-Y/TABLES/4
25. Fidock, D. A., Rosenthal, P. J., Croft, S. L., Brun, R., and Nwaka, S. (2004). Antimalarial drug discovery: efficacy models for compound screening. *Nature Reviews. Drug Discovery*, *3*(6): 509–520. https://doi.org/10.1038/NRD1416
26. Gheith, I., and El-Mahmoudy, A. Laboratory evidence for the hematopoietic potential of *Beta vulgaris* leaf and stalk extract in a phenylhydrazine model of anemia. *Brazilian Journal of Medical and Biological Research*, 2018; *51*(11): 1–8. https://doi.org/10.1590/1414-431X20187722
27. Godwin, E., Emmanuel, A., Onakpa, A. F., Wandayi, A., and Rhoda, O. A. Effect of the Aqueous Extracts of Alstonia boonei on the Haematological Profiles of Mice Experimentally Infected with the Chloroquine-Sensitive Strain of Plasmodium berghei NK-65. *Hematologia*, 2011; *1*(1): 11–18. https://doi.org/10.3923/HEMATOL.2012.11.18
28. Gramaglia, I., Sahlin, H., Nolan, J. P., Frangos, J. A., Intaglietta, M., and van der Heyde, H. C. Cell-Rather Than Antibody-Mediated Immunity Leads to the Development of Profound Thrombocytopenia during Experimental Plasmodium berghei Malaria. *The Journal of Immunology*, 2005; *175*(11): 7699–7707. https://doi.org/10.4049/JIMMUNOL.175.11.7699
29. Habluetzel, A., Pinto, B., Tapanelli, S., Nkouangang, J., Saviozzi, M., Chianese, G., Lopatriello, A., Tenoh, A. R., Yerbanga, R. S., Taglialatela-Scafati, O., Esposito, F., and Bruschi, F. Effects of Azadirachta indica seed kernel extracts on early erythrocytic schizogony of Plasmodium berghei and pro-inflammatory response in inbred mice. *Malaria Journal*, 2019; *18*(1): 1–9. https://doi.org/10.1186/S12936-019-2671-8/FIGURES/3
30. Haldar, K., and Mohandas, N. Malaria, erythrocytic infection, and anemia. *Hematology / the Education Program of the American Society of Hematology. American Society of Hematology. Education Program*, 2009; *1*: 87–93. https://doi.org/10.1182/ASHEDUCATION-2009.1.87
31. Inyang, A. L., Okpako, D. T., and Essien, E. M. Platelet reactions in acute Plasmodium berghei infection in Swiss albino mice. *Haematologia*, 1987; *20*(2): 101–108.
32. Islas, J. F., Acosta, E., G-Buentello, Z., Delgado-Gallegos, J. L., Moreno-Treviño, M. G., Escalante, B., and Moreno-Cuevas, J. E. An overview of Neem (Azadirachta indica) and its potential impact on health. *Journal of Functional Foods*, 2020; *74*: 104171. https://doi.org/10.1016/J.JFF.2020.104171
33. Jorum, H. . O., and Piero, M. N. Haematological Effects of Dichloromethane-Methanolic Leaf Extracts of Carissa edulis (Forssk.) Vahl in Normal Rat Models. *Journal of Hematology and Thromboembolic Diseases*, 2016; *04*(01): 1–8. https://doi.org/10.4172/2329-8790.1000232
34. Kabiru, A. Y., Ibikunle, G. F., Innalegwu, D. A., Bola, B. M., and Madaki, F. M. In Vivo Antiplasmodial and Analgesic Effect of Crude Ethanol Extract of Piper guineense Leaf Extract in Albino Mice. *Scientifica*, *2016*. https://doi.org/10.1155/2016/8687313
35. Karimi, A., Navidbakhsh, M., Haghi, A. M., and Faghihi, S. A morphology-based method for the diagnosis of red blood cells parasitized by Plasmodium malariae and Plasmodium ovale. *Scandinavian Journal of Infectious Diseases*, 2014; *46*: 368–375. https://doi.org/10.3109/00365548.2014.880186
36. Kotepui, M., Phunphuech, B., Phiwklam, N., Chupeerach, C., and Duangmano, S. Effect of malarial infection on haematological parameters in population near Thailand-Myanmar border. *Malaria Journal*, 2014; *13*(1): 1–7. https://doi.org/10.1186/1475-2875-13-218/TABLES/4
37. Kumar, V. S., and Navaratnam, V. Neem (Azadirachta indica): Prehistory to contemporary medicinal uses to humankind. *Asian Pacific Journal of Tropical Biomedicine*, 2013; *3*(7): 505–514. https://doi.org/10.1016/S2221-1691(13)60105-7
38. Kwon, S., Lee, D. H., Han, S. J., Yang, W., Quan, F. S., and Kim, K. S. Biomechanical properties of red blood cells infected by Plasmodium berghei ANKA. *Journal of Cellular Physiology*, 2019; *234*(11): 20546–20553. https://doi.org/10.1002/JCP.28654
39. Lorke, D. A new approach to practical acute toxicity testing. *Archives of Toxicology*, 1983; *54*(4): 275–287. https://doi.org/10.1007/BF01234480
40. Maji, S., and Modak, S. Neem: Treasure of Natural Phytochemicals. *Chemical Science Review and*

Letters, 2021; *10*(39): 396–401. https://doi.org/10.37273/chesci.cs205205351
41. Mohammadi, S., Jafari, B., Asgharian, P., Martorell, M., and Sharifi-Rad, J. Medicinal plants used in the treatment of Malaria: A key emphasis to Artemisia, Cinchona, Cryptolepis, and Tabebuia genera. *Phytotherapy Research*, 2020; *34*(7): 1556–1569. https://doi.org/10.1002/PTR.6628
42. Momoh, J., Longe, A. O., Aina, O. ., and Ajibaye, O. IN-VIVO ANTI-PLASMODIAL ACTIVITY AND IN-VITRO ANTIOXIDANT PROPERTIES OF METHANOLIC LEAF EXTRACT OF AZADIRACHTA INDICA AND ITS POSITIVE EFFECT ON HEMATOLOGICAL AND LIPID PARAMETERS IN SWISS ALBINO MICE INFECTED WITH PLASMODIUM BERGHEI NK. *European Scientific Journal*, *1*(February), 2015; 65: 456–467.
43. Moreno, M., and Wiegand, T. J. Blood. *Encyclopedia of Toxicology: Third Edition*, 2014; 526–532. https://doi.org/10.1016/B978-0-12-386454-3.00683-7
44. Ojo, O. A., Ojo, A. B., Oyinloye, B. E., Ajiboye, B. O., Anifowose, O. O., Akawa, A., Olaiya, O. E., Olasehinde, O. R., and Kappo, A. P. Ocimum gratissimum Linn. Leaves reduce the key enzymes activities relevant to erectile dysfunction in isolated penile and testicular tissues of rats. *BMC Complementary and Alternative Medicine*, 2019; *19*(1): 1–10. https://doi.org/10.1186/s12906-019-2481-0
45. Ojueromi, O. O., Oboh, G., and Ademosun, A. O. Effect of black seeds (Nigella sativa) on inflammatory and immunomodulatory markers in Plasmodium berghei-infected mice. *Journal of Food Biochemistry*, 2022; *46*(11): 1–27. https://doi.org/10.1111/jfbc.14300
46. Omarine Nlinwe, N., and Nange, T. B. Assessment of Hematological Parameters in Malaria, among Adult Patients Attending the Bamenda Regional Hospital. *Anemia*, 2020; *2020*: 3814513. https://doi.org/10.1155/2020/3814513
47. Omoirri MA, Madubogwu NU, Iloh SE, Adejumo SA, and Ojiako CM. Renal architectural changes in Plasmodium berghei inoculated mice. *GSC Biological and Pharmaceutical Sciences*, 2020; *13*(3): 222–227. https://doi.org/10.30574/gscbps.2020.13.3.0409
48. Osonwa, U., Eluu, S., and Oli, A. Antiplasmodial and Biochemical Effects of Combination of Ethanolic Leave-extracts of Azadirachta indica and Ocimum gratissimum on Plasmodium berghei-infected Mice. *African Journal of Pharmaceutical Sciences and Pharmacy*, 2017; *5*: 15–29.
49. Pandey, S. (2017). Phytochemical constituents, pharmacological and TRADITIONAL USES OF OCIMUM GRATISSIMUM L IN TROPICS. *IAJPS J. P. Sci*, *2017; 4*(411): 4234–4242. https://doi.org/10.5281/zenodo.1056925
50. Rudrapal, M., and Chetia, D. Malaria and Recent Developments in Antimalarial Drugs. *Neglected Tropical Diseases and Phytochemicals in Drug Discovery*, 2021; 499–542. https://doi.org/10.1002/9781119617143.CH21
51. Sadi, D., and Imam, T. S. Effect of Cymbopogon citratus (lemon grass) leaves extract on haematological and biochemical parameters in albino rats (Rattus norvegicus). *Katsina Journal of Natural and Applied Sciences*, 2019; *8*(1): 183–189. https://www.researchgate.net/publication/354792224
52. Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., and Mann, A. Scientific basis for the therapeutic use of Cymbopogon citratus, stapf (Lemon grass). *Journal of Advanced Pharmaceutical Technology and Research*, 2011; *2*(1): 3. https://doi.org/10.4103/2231-4040.79796
53. Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., and Mann, A. S. Scientific basis for the therapeutic use of Cymbopogon citratus, stapf (Lemon grass). *Journal of Advanced Pharmaceutical Technology and Research*, 2011; *2*(1): 3–8. https://doi.org/10.4103/2231-4040.79796
54. Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., and Mann, A. S. Scientific basis for the therapeutic use of *Cymbopogon citratus*, stapf (Lemon grass). *Journal of Advanced Pharmaceutical Technology and Research*, 2011; *2*(1): 3–8. https://doi.org/10.4103/2231-4040.79796
55. Shija, K. M., Nondo, R. S. O., Mloka, D., Sangeda, R. Z., and Bwire, G. M. Effects of lemon decoction on malaria parasite clearance and selected hematological parameters in Plasmodium berghei ANKA infected mice. *BMC Complementary Medicine and Therapies*, 2020; *20*(1): 24. https://doi.org/10.1186/S12906-020-2820-1
56. Shittu, S.-T., Oyeyemi, W., Lasisi, T., Shittu, S.-S., Lawal, T., and Olujobi, S. Aqueous leaf extract of Ocimum gratissimum improves hematological parameters in alloxan-induced diabetic rats via its antioxidant properties. *International Journal of Applied and Basic Medical Research*, 2016; *6*(2): 96–100. https://doi.org/10.4103/2229-516X.179016
57. Sowunmi, A., Gbotosho, G. O., Adedeji, A. A., Fateye, B. A., Sabitu, M. F., Happi, C. T., and Fehintola, F. A. Effects of acute Plasmodium falciparum malaria on body weight in children in an endemic area. *Parasitology Research*, 2007; *101*(2): 343–349. https://doi.org/10.1007/S00436-007-0494-Y
58. Sujarwo, W., Keim, A. P., Caneva, G., Toniolo, C., and Nicoletti, M. Ethnobotanical uses of neem (Azadirachta indica A.Juss.; Meliaceae) leaves in Bali (Indonesia) and the Indian subcontinent in relation with historical background and phytochemical properties. *Journal of Ethnopharmacology*, 2016; *189*: 186–193. https://doi.org/10.1016/J.JEP.2016.05.014
59. Syukriah, S., Faizah, U. N., Saragih, H. T. S., Yuneldi, R. F., Poerwanto, S. H., Astuti, R. R. U. N. W., and Immenschuh, S. The Oxidative Stress-

Mediated Effects in Pregnant Mice with Plasmodium berghei Infection. *Advances in Animal and Veterinary Sciences*, 2022; *10*(3): 521–528. https://doi.org/10.17582/JOURNAL.AAVS/2022/10.3.521.528

60. Tajbakhsh, E., Kwenti, T. E., Kheyri, P., Nezaratizade, S., Lindsay, D. S., and Khamesipour, F. Antiplasmodial, antimalarial activities and toxicity of African medicinal plants: a systematic review of literature. *Malaria Journal*, 2021; *20*(1): 1–50. https://doi.org/10.1186/S12936-021-03866-0
61. Toma, A., Deyno, S., Fikru, A., Eyado, A., and Beale, A. In vivo antiplasmodial and toxicological effect of crude ethanol extract of Echinops kebericho traditionally used in treatment of malaria in Ethiopia. *Malaria Journal*, 2015; *14*(1): 196. https://doi.org/10.1186/S12936-015-0716-1
62. Ukpai, O. M., and Amaechi, E. C. Evaluation of In Vivo Antimalarial Activity of The Ethanolic Leaf Extracts of Chromolaena Odorata and Cymbopogon Citratus In Mice. *Nigerian Journal Of Biotechnology*, 2012; *24*(1): 27–34.
63. Wang, H., Li, S., Cui, Z., Qin, T., Shi, H., Ma, J., Li, L., Yu, G., Jiang, T., and Li, C. Analysis of spleen histopathology, splenocyte composition and haematological parameters in four strains of mice infected with Plasmodium berghei K173. *Malaria Journal*, 2021; *20*(1): 249. https://doi.org/10.1186/S12936-021-03786-Z
64. White, N. J. Anaemia and malaria. *Malaria Journal*, 2018; *17*(1): 371. https://doi.org/10.1186/S12936-018-2509-9
65. White, N. J., Pukrittayakamee, S., Hien, T. T., Faiz, M. A., Mokuolu, O. A., and Dondorp, A. M. Malaria. *Lancet (London, England)*, 2014; *383*(9918): 723–735. https://doi.org/10.1016/S0140-6736(13)60024-0